International Journal of Dental Research & Development (IJDRD)
ISSN(P): 2250-2386; ISSN(E): 2321-0117
Vol. 6, Issue 4, Aug 2016, 49-58

TRANS STELLAR
•Journal Publications •Research Consultancy

A CURSORY GLANCE ON BIOMARKERS FOR BONE IN HEALTH AND DISEASE

MEHAK KAUL[1], SAGRIKA SHUKLA[2] & SURESH D.K[3]

[1]*Reaserch Scholar, Department of Periodontology, Seema Dental College and Hospital, Rishikesh, Uttarakhand, India*

[2]*Faculty, Department of Periodontology, Seema Dental College and Hospital, Rishikesh, Uttarakhand, India*

[3]*Professor and Head, Department of Periodontology, Seema Dental College and Hospital, Rishikesh, Uttarakhand, India*

ABSTRACT

Periodontitis, an inflammatory disorder is characterized by soft tissue as well as hard tissue destruction. Biochemical markers of bone remodeling provide clinically useful evidence of the normal and pathologic processes that reflect bone cell activity in the periodontium. There is this major challenge always in clinical periodontics to find a reliable molecular marker of periodontal tissue destruction with high sensitivity, specificity and utility. This review article enumerates various biomarkers responsible for bone destruction whose presence and absence can guide a clinician with the periodontal disease activity taking place.

KEYWORDS: Biomarkers, Bone Formation, Bone Remodeling, Periodontitis

Received: Jul 10, 2016; **Accepted:** Aug 01, 2016; **Published:** Aug 12, 2016; **Paper Id.:** IJDRDAUG20167

Original Article

INTRODUCTION

Periodontitis is a plethora of various inflammatory processes which is characterized by periodontal pocket formation and alveolar bone resorption eventually followed by tooth loss.[1] As soon as the initiation of the disease takes place, periodontitis progresses with the degradation of collagen fibers and their attachment to the cemental surface with the apical migration of the pocket epithelium ultimately forming deep periodontal pockets, along with the resorption of alveolar bone.[2,3]

The periodontal diagnosis should not only aim at mere diagnosis of the underlying disease but should also, serve as a basis for planning proper treatment and providing means for assessing the effectiveness of the specified periodontal therapy. Always in the diseased tissues, biochemical signaling takes place which involves three biological phases; inflammation, connective tissue degradation, and alveolar bone turnover contributing to the clinical morbidity. Elevated levels of these circulating molecules have been evaluated in gingival crevicular fluid, serum and whole saliva of patients affected with periodontitis making them putative biomarkers of the disease.[4] Various biochemical markers of bone remodeling which include markers for bone formation and bone resorption are currently in use. These markers act as useful evidence for the normal and pathological processes that reflect bone cell activities in the skeleton. Bone remodelling markers can be used to document the effects of various therapeutic agents in patients with bone diseases and possibly reduce the need for frequent bone density testing.[5] Biomarkers responsible for bone remodeling can be either resorption markers showing osteoclastic activity or formation markers showing osteoblastic activity. Both these activities are coupled and any change in the level of these markers reflects change in the bone turnover.[6]

Ever since last decade bone markers are increasingly being used for the therapeutic monitoring of patients with metabolic bone diseases. However, large trials are required so that these measurements can be useful for consistent and reproducible results.[7,8]

WHAT IS A BIOMARKER?

A biomarker is defined as a substance that clear indicates a biologic state. It can be defined as, a substance that is measured objectively and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[9]

In order to better understand the biochemical processes in bones and isolation and characterization of cellular components of the skeletal matrix, the number of new potential biochemical markers of bone formation and resorption are under constant study.

CLASSIFICATION OF POSSIBLE BIOMARKERS: [10]

Enzymes: Alkaline phosphatase, Aminopeptidase, Trypsin, á glucosidase, â galactosidase, â glucosidase, â glucuronidase, Gelatinase, Esterase, Collagenase, Kininase

Immunoglobulin: Ig A, Ig G, Ig M, sIg -A

Protein: Cystatin, Fibronectin, Lactoferrin, Vascular endothelial growth factors, Platelet activating factors, epidermal growth factors

Phenotypic marker: Epithelial keratin

Host cell: Leukocytes (PMN's)

Ion: Calcium

Hormones: Cortisol

Bacteria: A.a, P. gingivalis, P. intermedia, P. micros, C. rectus, T. denticola, B. forsythus, P. micros, mycoplasma

Volatile compounds: Hydrogen sulfide, Methyl mercaptan, Picolines, Pyridines

IMMUNE ROLEPLAY NECESSITATING THE NEED OF BIOMARKERS IN ORAL DISEASES

Under physiological conditions, bone is periodically resorbed by osteoclasts while new bone is formed by osteoblasts.[11] The discovery of RANKL and its decoy receptor, osteoprotegerin confirmed the idea that differentiation and function of osteoclasts are regulated by osteoblasts.[11] The recruitment of new osteoclasts is dependent on the balance between RANKL and osteoprotegerin in osteoblasts. In inflammatory bone resorption, however, activated T lymphocytes might mediate bone resorption through excessive production of soluble RANKL. RANKL is a membrane-anchored protein in osteoblasts, but the expression of membrane-anchored RANKL on T cells is limited and the majority of RANKL protein produced by T cells may be active in the soluble form after shedding. [12]

In a study conducted by **Teng et al.**[13] on patients with aggressive periodontitis infected with A. actinomycetemcomitans, T lymphocytes were isolated which expressed RANKL in response to A. actinomycetemcomitans. Immunodeficient mice orally infected with A. actinomycetemcomitans were reconstituted with the T lymphocytes from the patients by adoptive transfer. Adoptive transfer of T cells from periodontitis patients caused

severe bone destruction and the bone resorption was suppressed by osteoprotegerin, suggesting that the destruction was mediated by soluble RANKL produced from the transferred T cells. In another study by **Choi et al.**[14] it was reported that B lymphocytes also produce RANKL and augment osteoclastogenesis.

Henceforth, it becomes important to understand the interaction between stromal cells (osteoblasts, periodontal ligament fibroblasts, and gingival fibroblasts), inflammatory infiltrates (T and B lymphocytes and macrophages) and various inflammatory mediators, including IL-1, IL-6, tumor necrosis factor-a and prostaglandin E2, which can induce bone resorption indirectly by stimulating osteoblasts to produce RANKL. [15] Enhanced RANKL production might be associated with alveolar bone resorption, and lymphocytes are one of the major RANKL-expressing cells in periodontitis tissue.[16]

It has been a great challenge to determine biomarkers for screening, prognosis and evaluating the disease activity and the efficacy of therapy (diagnostic tests) when diagnosing the oral diseases and conditions. An oral diagnostic tool, in general, should provide accurate information for differential diagnosis, localization of disease and severity of infection. Traditional diagnostic measures, such as visual examination, tactile appreciation, periodontal pocket depth, attachment level, and plaque index, bleeding on probing and radiographic assessment of alveolar bone loss are still popular and universally used. Saliva is simple, non-invasive, readily available and easily collected without specialized equipment or personnel. For the past two decades, saliva has been increasingly evaluated as a diagnostic fluid for detecting various diseases affecting human population like breast cancer, oral cancer, caries risk, salivary gland diseases, periodontitis, and systemic disorders such as hepatitis C and the presence of human immunodeficiency virus (HIV). It reflects various markers for different kinds of diseases which may be of different etiologies like hormonal, therapeutic, immunological and neoplastic. Various mediators of chronic inflammation and tissue destruction have been detected in whole saliva of patient with oral diseases. Salivary biomarkers have also been used to examine the effect of lifestyle factors, including smoking, on periodontal health.[9]

CRITERIAS IMPORTANT FOR MEASURING BONE REMODELING MARKERS:[17]

- **Biological Factors** include tissue specificity, factors which determine the effect of change in liver or kidney function on marker clearance, the biological rhythm of the marker due to standardization of physiological sampling time, immobilization, etc.
- **Preanalytical Factors** include sample storage procedures, i. e. time and temperature, sample freezing and thawing, anticoagulant effect, etc.
- **Analytical Specificity and Accuracy** include microheterogeneity of markers, also the possibility of marker resorption into several different fragments, specificity of antibodies and, enzyme activity inhibitors.
- **Diagnostic Validity** include the question of differences between markers considering their diagnostic sensitivity and specificity.

GENERAL SIGNIFICANCE OF BIOCHEMICAL MARKERS OF BONE REMODELING

The basic advantage of biochemical markers, over bone mineral densitometry and bone biopsy, is that biomarkers give the entailed information about the status of bone remodeling. In addition, biochemical markers are noninvasive and are early indicator of some pathological changes in bones or the risk of some bone disease. There are significant changes in

biochemical markers after 1 to 3 months of effective therapy while bone mass changes can be adequately evaluated only after the 1st or rather the 2nd year.[18,19] It has been shown so far without doubt that some of the markers or marker combinations are quite useful in monitoring of anti resorption therapy effect. The final assessment of their clinical usefulness in patient management is still under way. Therefore, it is still recommended to pay maximum attention to the issue of whether and how the markers are to be used and to standardization of pre analytical and analytical procedures of bone marker determination.[5]

VARIOUS BIOMARKERS RESPONSIBLE FOR BONE FORMATION (TABLE 1)

Osteoblasts are mononuclear cells that attach to bone surfaces and form new bone. They are most commonly located at sites that recently underwent resorption. They produce type I collagen and other matrix components of osteoid, and they also mineralize the osteoid with hydroxyapatite.[20] Growing children have much more osteoblasts as compared to adults. In elderly women due to estrogen deficiency, osteoblasts may increase in number in response to the increase in bone resorption.[21] In elderly men, osteoblast activity may decrease, possibly because of decreasing levels of serum insulin-like growth factor 1 and testosterone.[22,23] Osteonectin is a single chain polypeptide that binds strongly to hydroxyapatite and other extracellular matrix proteins.[6]

VARIOUS BIOMARKERS RESPONSIBLE FOR BONE RESORPTION (TABLE 2)

Osteoclasts are multinucleated cells that resorb bone. They initiate bone remodeling and help shape growing bone and so are more numerous in children.[30] Bone resorption markers are secreted in urine, they were mostly measured in urine until recently; variability in the results led to the significant change. Therefore, the main scientific and commercial interest has been focused on setting up and evaluating methods for their measuring in serum.[3]

VARIOUS FACTORS INFLUENCING BIOMARKER LEVELS

Various factors influence the results of assay and these factors are enlisted as under:

- **Diurnal and Day-to-Day Variability**

It is essential to know that the levels of bone turnover markers are highest in the early morning and lowest in the afternoon and evening. Also, the levels of urinary markers can vary 20% to 30% from the highest to lowest value of the day.[34] The serum markers of bone formation appear to vary less from day to day.[20]

- **Calcium Intake**

An increase in calcium intake also can lower the levels of bone resorption markers, particularly in people whose calcium intake was previously low. Presumably, this effect is mediated by inhibition of parathyroid hormone secretion.[35]

- **Sample Handling**

Improper collection and handling of specimens can seriously affect the assay precision. The optimal time to collect samples is in the morning. It is also important to use the same laboratory for serial measurements, since assay results can vary considerably among laboratories, even if they use identical methods.[20]

CLINICAL APPLICATIONS OF BIOMARKERS

- **Postmenopausal Osteoporosis**

Markers of bone formation are elevated than markers of bone resorption in such patients, and if they are elevated, they decrease as expected in response to therapy that inhibits bone resorption, though more gradually and to a lesser extent than the resorption markers.[36,37]

- **Primary Hyperparathyroidism**

Hyperparathyroidism patients exhibit high levels of markers of bone resorption and formation. [36,37]

- **Osteomalacia and Rickets**

Bone resorption markers are elevated in vitamin D deficiency and this eventually determines the response to therapy.[38]

DISCUSSIONS

Progressive periodontitis needs to be early diagnosed and treated as the destructuction caused by this will lead to tooth loss and soft tissue anatomy destruction. Development of biomarker kits and tests provide useful information to the clinician regarding the present periodontal disease type, location and severity. These methods are optimal and appropriately determine the presence of current disease activity, predict sites for future breakdown and assess the response to periodontal intervention. Bone biomarkers of disease play an important role in life sciences and have begun to assume a greater role in diagnosis, monitoring of the disease activity and prediction of the progress of the treatment.[39]

Alkaline phosphatase which is a membrane bound glycoprotein and is mainly involved in the maintenance of alveolar bone and renewal of the periodontal ligament. In one of the studies conducted by **Gibert et al,** [40] serum levels of ALP from patients with the chronic periodontal disease were evaluated and compared with control patients. It was observed that there was a positive relationship between attachment loss in the periodontal group and a drop in ALP activity in serum. Hence, it was concluded that ALP might serve as a marker in periodontal treatment planning and monitoring.

Osteocalcin levels have been found elevated during in diseases such as osteoporosis, multiple myelomas, and fracture repair. **Kunimatsu et al.** [41], reported association between GCF osteocalcin, N terminal peptide levels and various clinical parameters in a cross sectional study of patients with periodontitis and gingivitis. **Nakashima et al.**[42], reported presence of significant levels of GCF osteocalcin from both periodontitis and gingivitis patients. Osteocalcin levels were also significantly correlated with pocket depth and gingival index scores, as well as GCF levels of ALP and PGE2.

Osteopontin(OPN) is produced by both osteoclasts and osteoblasts. It exhibits dual function in bone maturation and mineralization and also linked with bone resorption. GCF OPN secretion increased proportionally with the progression of disease and with nonsurgical treatment it was significantly reduced.[42] Given the specificity and sensitivity for bone resorption, C-Telopeptide pyridinoline cross linked type I collagen (ICTP) represent a potentially valuable diagnostic aid for periodontal disease. It is useful in differentiating between the presence of gingival inflammation and active periodontal or perimplant bone destruction. ICTP has been shown to be a promising predictor of both future alveolar bone and attachment loss.[43] ICTP levels strongly correlated with whole subject level of several periodontal pathogens including T forsythsis, P gingivalis and T denticola.

Whenever diagnosis is made only on periodontal probing measurement chances of inappropriate therapeutic intervention and disease management are likely to happen. The initiation and progression of periodontitis is associated with various risk factors and these factors are solely responsible for causing changes in biomarker concentration related to the disease. Various diagnostic and prognostic tests need to be evaluated and developed so that the disease progression can be intercepted at right times.

Various methods have been developed that identify the risk factors associated with the disease and measure various biomarkers out of which bone biomarkers hold a significant importance in detection of periodontitis.[6] methods and the latest diagnostic kits hold importance but also exhibit certain limitations. Traditional methods which includes the testing of salivary, urinary, GCF and blood samples to evaluate the levels of biochemical markers of bone turnover have proved to be useful, non-invasive and a relatively inexpensive tool for studying bone metabolism, however, these techniques provide information about past disease activity and are unable to diagnose the present disease activity.[45] The development of new markers of bone turnover will increase the knowledge of pathophysiology of periodontitis and metabolic bone diseases like osteoporosis. After further evaluation, these markers may find a place in the clinical case of postmenopausal women. Microarray and Microfluidics are the recent treatment strategies that are made available for assessing the risk factors associated with the disease and also screen the presence of various essential biomarkers. Electrochemical biosensors coupled to Magnetic Beads are used for the detection of clinical biomarkers. These recent advances in biomarkers and diagnostic tools could prove a way better treatment approaches to periodontal disease.[6] Table 3 enumerates various studies conducted over a brief period of time on various bone remodeling biomarkers present in various periodontal diseases.

CONCLUSIONS

To summarize the above mentioned, there strives a need for long lasting longitudinal studies of biochemical bone remodeling markers on large population with different age and gender which correlates to reference procedures of bone mass measurement. Furthermore, validation of evaluation of these biomarker usage and their association with the disease will be required to monitor the treatment plan for the disease. combination of genetic and biochemical markers in risk assessment of osteoporosis and other bone diseases is a great challenge in itself and studies for this should be taken into consideration so that a new benchmark is set for a proper diagnosis of the underlying disease and its treatment.

REFERENCES

1. *Nagasawa, T. et al. (2007). Roles of receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin in periodontal health and disease. Periodontology 2000, 43, 65-84.*
2. *Ellison, S.A. (1970). Oral bacteria and periodontal disease. Dental Research Journal, 49,198–99.*
3. *Genco, R.J. (1969). A Review of Dental Reasearch in microbiology with emphasis on periodontal disease. American Dental Association Journal, 78, 1016–36.*
4. *Taba, M. et al.(2005). Diagnostic Biomarkers for Oral and Periodontal disease. Dental Clinics of North America, 49,551–71.*
5. *Cepelak, I., Cvoriscec, D. (2009). Biochemical markers of bone remodeling – review. Biochemia Medica, 19, 1735-43.*
6. *Ram, V.S., Parthiban, Sudhakar, U., Mithradas, N. (2015). Bone biomarkers in Periodontal disease: A review article. Journal of Clinical and Diagnostic Research, 9, 1-7.*

7. *Woitge, H.W., Seibel, M.J. (1999). Molecular markers of bone and cartilage metabolism. Current Opinion in Rheumatology, 11,218–25.*
8. *Seibel, M.J. et al. (1999). Quantifiable manifestations of age-related bone loss. In: Rosen C.R, Glowacki J, Bilezikian J.P, eds. The aging skeleton. San Diego: Academic Press, 277–300.*
9. *Khashu, H., Baiju, C.S., Bansal, S.R., Chhillar, A. (2012). Salivary Biomarkers: A Periodontal Overview. Oral Health and Community Dental Journal, 6, 28-33.*
10. *Atkinson, A.J. et al. (2001). Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clinical Pharmacology and Therapeutics Journal, 69, 89–95.*
11. *Withold, W. (1996). Monitoring of bone turnover biological, preanalytical and technical criteria in assessment of biochemical markers. European Journal of Clinical Chemical and Clinical Biochemistry, 34, 785-99.*
12. *Suda, T. et al. (1999). Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocrinology Review, 20, 345–57.*
13. *Kanamaru, F. et al. (2004). Expression of membrane-bound and soluble receptor activator of NF-kappaB ligand (RANKL) in human T cells. Immunology Letters Journal, 94,239–46.*
14. *Teng, Y.T. et al. (2000). Functional human T-cell immunity and osteoprotegerin ligand control alveolar bone destruction in periodontal infection. Journal of Clinical Investigation, 106,59–67.*
15. *Choi, Y. et al. (2001). Osteoclastogenesis is enhanced by activated B cells but suppressed by activated CD8 (+) T cells. European Journal of Immunology, 31, 2179–88.*
16. *Taubman, M.A., Kawai, T. (2001). Involvement of T-lymphocytes in periodontal disease and in direct and indirect induction of bone resorption. Critical Reviews in Oral Biology and Medicine, 12, 125-35.*
17. *Hormdee, D. et al. (2005). Protein kinase- A-dependent osteoprotegerin production on IL-1 stimulation in human gingival fibroblasts is distinct from periodontal ligament fibroblasts. Clinical and Experimental Immunology, 142, 490–97.*
18. *Engler, H. et al. (1998). Diagnostic and prognostic value of biochemical markers in malignant bone disease: A prospective study on the effect of biphosphonate on pain intensity and progression of malignant bone disease. Clinical Chemistry and Laboratory Medicine, 36, 879-85.*
19. *Demers, L.M. (1997). Clinical usefulness of markers of bone resorption and formation. Scandanavian Journal of Clinical and Laboratory Investigation, 57, 12-20.*
20. *Singer, F.R., Eyre, D.R. (2008). Using biochemical markers of bone turnover in clinical practice. Cleveland Clinic Journal of Medicine, 75,739-50.*
21. *Glorieux, F.H. et al. (2000). Normative data for iliac bone histomorphometry in growing children. Bone, 26,103–9.*
22. *Clarke, B.L. et al. (1996). Changes in quantitative bone histomorphometry in aging healthy men. Journal of clinical endocrinolology and metabolism, 81, 2264–70.*
23. *Rucker, D. et al. (2004). IGF-I and testosterone levels as predictors of bone mineral density in healthy, community-dwelling men. Clinical Endocrinology (Oxf)., 60,491–99.*
24. *Seibel, M.J., Woitge, W.H. (1999). Biochemical markers of bone metabolism Update 1999; Part I: Basic principles. Clinical Laboratory, 45,237-56.*
25. *Seibel, M.J. (2005). Biochemical markers of bone turnover Part I: Biochemistry and variability. Clinical Biochemist Reviews,*

26, 971-22.

26. *Delmas, P.D. (1999). Biochemical markers of bone turnover in Paget's disease of bone. Journal of Bone and Mineral Research, 14, 66-9.*
27. *Eastell, R., Baumann, M. et al (2001). Bone Markers: Biochemical and Clinical Perspectives. Martin Dunitz Ltd, The Livery House, 66-7.*
28. *M, Nakamura., J, Slots. (1983). Salivary enzymes. Origin and relationship to periodontal disease. Journal of Periodontal Research, 18,559-69.*
29. *Lian, J.B., Gundberg, C.M. (1988). Osteocalcin. Biochemical considerations and clinical applications. Clinical Orthopaedics and Research, 226,267-91.*
30. *Glorieux, F.H. et al. (2000). Normative data for iliac bone histomorphometry in growing children. Bone, 26,103–9.*
31. *Hannon, R. et al. (2004). Clinical performance of immunoreactive tartrate-resistant acid phosphatase isoform 5b as a marker of bone resorption. Bone, 34,187–94.*
32. *Meier, C. et al. (2006). Serum cathepsin K concentrations reflect osteoclastic activity in women with postmenopausal osteoporosis and patients with Paget's disease. Clinical Laboratory, 52, 1–10.*
33. *Boyce, B.F., Xing, L. (2007). Biology of RANK, RANKL, and Osteoprotegerin. Arthritis Research and Therapy, 9, 1.*
34. *Wichers, M., Schmidt, E. et al. (1999). Diurnal rhythm of CrossLaps in human serum. Clin Chem, 45, 1858-60.*
35. *Kenny, A.M. et al. (2004). Comparison of the effects of calcium loading with calcium citrate or calcium carbonate on bone turnover in postmenopausal women. Osteoporosis International, 15, 290–94.*
36. *Liberman, U.A. et al. (1995). Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis.*
37. *Black, D.M. et al. (2007). Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. New England Journal of Medicine, 356,1809–22.*
38. *Need, A.G. (2006). Bone resorption markers in vitamin D insufficiency. Clinica Chimica Acta, 368, 48–52.*
39. *Sanikop, S. et al. (2010). Gingival crevicular fluid alkaline phosphatase as a potential diagnostic marker of periodontal. Journal of Indian Society of Periodontology, 16,513-88.*
40. *Gibert, P. et al. (2003). Alkaline phosphatase isozyme activity in serum from patients with chronic periodontitis. Journal of Periodontol Research,38,362-65.*
41. *Kunimatsu, K. et al. (1993). A crossectional study on osteocalcin levels in gingival crevicular fluid from periodontal patient. Journal of Periodontology, 64,865-69.*
42. *Nakashima, K. et al. (1996). A longitudinal study of various crevicular fluid components as markers of periodontal disease activity. Journal of Clinical Periodontology, 23,832-38.*
43. *Charles, P. et al (1994). Assessment of bone remodelling using biochemical indicators of type I collagen synthesis and degradation. Relation to calcium kinetics. Bone and mineral, 37,805-10.*
44. *Gloria, A. et al (2013). Screening for salivary levels of deoxypyridinoline and bone specific alkaline phosphatase during orthodontic tooth movement: a pilot study. European Journal of Orthodontics, 35,365-68.*
45. *Pavankumar, A. et al. (2015). Biomarkers in periodontal disease. Journal of Molecular Biomarker Diagnosis, 6, 1-6.*

46. *Bostanci, N. et al. (2007). Gingival crevicular fluid levels of RANKL and OPG in periodontal diseases: implications of their relative ratio. Journal of Clinical Periodontology, 34,370–76.*
47. *Mogi, M. et al. (2004). Differential expression of RANKL and osteoprotegerin in gingival crevicular fluid of patients with periodontitis. Journal of Dental Research, 83,166–69.*
48. *Liu, D. et al. (2003). Expression of RANKL and OPG mRNA in periodontal disease: possible involvement in bone destruction. International Journal of Molecular Medicine, 11,17–21.*
49. *Vernal, R. et al. (2004). Levels of cytokine receptor activator of nuclear factor kappa B ligand in gingival crevicular fluid in untreated chronic periodontitis patients. Journal of Periodontology, 75, 1586–91.*
50. *Garg, G. et al. (2009). Effect of nonsurgical periodontal therapy on crevicular fluid levels of Cathepsin K in periodontitis. Archvies of Oral Biology, 54, 1046–51.*
51. *Otogoto, J. et al. (2007). Expression of cathepsin-K in gingival crevicular fluid of patients with periodontitis. Archives of Oral Biology, 52,894–98.*
52. *Frodge, B.D. et al. (2008). Bone remodeling biomarkers of periodontal disease in saliva. Journal of Periodontology, 79, 1913–19.*
53. *Kinane, D.F. et al. (2007). Host-response: understanding the cellular and molecular mechanisms of host-microbial interactions–consensus of the Seventh European Workshop on Periodontology. Journal of Clinical Periodontology, 38, 44–8.*
54. *Golub, L.M. et al. (1997). A matrix metalloproteinase inhibitor reduces bone-type collagen degradation fragments and specific collagenases in gingival crevicular fluid during adult periodontitis. Inflammation Research, 46,310–19.*

APPENDICIES

Table 1: Various Biomarkers Responsible For Bone Formation

Biomarker	Tissue Origin	Analytical Sample	Function
Total Alkaline Phosphatase Bone Alkaline Phosphatase	Bone, Liver[24,25] Bone[24,25]	Serum[27] Serum[27]	Normal turnover of PDL, Root cementum formation and maintenance, and bone homeostasis[27,28]
Osteocalcin	Bone, Trombocytes[26,27]	Serum[27]	Influences energy metabolism by modulating the production and action of insulin[27,29]
C-Terminal propeptide of type-I procollagen (PICP)	Bone, Skin, Soft tissues[27]	Serum[27]	Proliferation of osteoblasts and fibroblasts[27]

Table 2: Various Biomarkers Responsible For Bone Resorption

Biomarker	Tissue Origin	Analytical Sample	Function
Pyridinoline (PYD)	Bone, Tendon, Cartilage [24,25]	Urine[27]	Useful in differentiating the presence of gingival inflammation from active periodontal and periimplant bone destruction.[6]
Deoxypyridinoline (DPD)	Bone[24,25]	Serum[27]	Does not metabolize before secretion into urine[27]

Table 2: Contd.,			
Cross-Linked Telopeptides • **NTx** • **CTx**	Bone[27] Bone[27]	Urine and Serum[27] Urine and Serum[27]	Osteoclastic proteolysis[27]
Serum tartrate-resistant acid phosphatase (TRAP) 5b	Bone[27]	Serum[27]	Correlates with other markers of bone resorption[31]
Serum cathepsin K	Bone[27]	Serum[27]	Degrades bone type I collagen during resorption[32]
Receptor Activator of Nuclear Factor Kappa (RANK)	Bone[27]	Serum[27]	Regulator of osteoclast recruitment and activity[33]

Table 3: Various Studies Enumerating Various Bone Remodeling Biomarkers in Different Periodontal Diseases

Studies	Biomarker	Sample	Disease
Talonpoika et al[61](1994)	cross-linked N-terminal telopeptide(NT_x)	GCF	Chronic Periodontitis
Golub et al[54] (1997)	ICTP	GCF	Chronic Periodontitis
Palys et al[58] (1998)	ICTP	GCF	Gingivitis
Oringer et al[54] (2002)	ICTP	GCF	Chronic Periodontitis
Liu et al [48] (2003)	RANK, OPG	GCF	Aggressive Periodontitis
Vernal et al [49] (2004)	RANKL	GCF	Chronic Periodontitis
Mogi et al [47] (2004)	RANKL, OPG	GCF	Aggressive Periodontitis
Otogoto et al [51](2007)	Cathepsin K	GCF	Chronic Periodontitis
Bostanci et al [46] (2007)	RANKL,OPG	GCF	Aggressive Periodontitis
Kinane et al [53](2007)	ICTP	Saliva	Chronic Periodontitis
Frodge et al[52] (2008)	ICTP, RANKL	Saliva	Chronic Periodontitis
Buduneli et al[55] (2008)	RANKL, OPG	Saliva	Chronic Periodontitis
Sakellari et al[59] (2008)	RANKL	GCF	Chronic Periodontitis
Garg et al[50] (2009)	Cathepsin K	GCF	Aggressive Periodontitis
Gurlek et al[56] (2009)	ICTP, Osteocalcin	Saliva	Chronic Periodontitis
Ozcaka et al[57](2010)	ICTP, Osteocalcin	Plasma	Chronic Periodontitis
Becerik et al[60] (2011)	Calprotectin, Osteocalcin and cross-linked N-terminal telopeptide(NT_x)	GCF	Aggresive Periodontitis and Chronic Periodontitis
Ganganna[62] (2016)	cross-linked N-terminal telopeptide(NTx)	Plasma	Chronic Periodontitis

GCF= Gingival Crevicular Fluid, ICTP= C-Telopeptide pyridinoline cross linked type I collagen